An Improved Method for Detecting Viruses in Water

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Enteroviruses, such as echovirus and coxsackievirus, have been implicated in numerous waterborne disease outbreaks of gastroenteritis worldwide. However, comprehensive occurrence studies of enteroviruses in drinking water matrices are limited, in part because of the lack of available methods that are rapid, sensitive, and able to detect live infectious virus. To address this issue, the US EPA has included echoviruses and coxsackieviruses on the microbial contaminant candidate list (CCL). To provide the research needed to support regulation decisions for these CCL viruses, our efforts have focused on developing methods for the rapid detection of live virus. The methods currently available for virus detection focus on either cell culture assays or molecular-based approaches. Cell culture–based assays can detect viable virus, but require several days or even weeks to complete. In contrast, molecular assays can rapidly detect virus genomic material, but do not determine the infectivity of the virus. To design a complete method for the detection of live infectious enterovirus, we recently developed a method which integrates cell culture and molecular assays. The method involves the filtration of virus from 200L of water and the concentration of the eluate into smaller volumes for inoculation into a cell line. The presence of viral genomic material is detected by quantitative real-time reverse transcription polymerase chain reaction (qRT-PCR). The viability of the detected virus is determined by observing increases in the genomic material over the course of the experiment, which is due to virus replication within the cell culture. Our results reveal that this method can detect as little as two infectious virus units in reagent water in less than 48 hours. In environmental water samples, six infectious virus units can be detected in 24 hours of growth in culture. This compares to the 4–8 days required to detect these viruses by cell culture alone. Our results demonstrate that incorporating a qRT-PCR step allows the detection of live infectious virus before infection is visible in cell culture alone. Moreover, when used to detect virus in environmental samples, this approach minimizes loss of virus compared with previous methods, thus greatly increasing the sensitivity of this approach.

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